CLAIMS

1. A polynucleotide encoding a polypeptide involved in spiramycin biosynthesis, wherein the sequence of said polynucleotide is:

(a) one of the sequences SEQ ID No. 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 28, 30, 34, 36, 40, 43, 45, 47, 49, 53, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 107, 109, 111, 113, 115, 118, 120, 141, 143, 145, 147 and 149,

(b) one of the sequences consisting of the variants of the sequences (a),

(c) one of the sequences derived from the sequences (a) and (b) due to the degeneracy of the genetic code.

2. A polynucleotide which hybridizes, under high stringency conditions, with at least one of the polynucleotides as claimed in claim 1.

3. A polynucleotide exhibiting at least 70%, 80%, 85%, 90%, 95% or 98% nucleotide identity with a polynucleotide comprising at least 10, 12, 15, 18, 20 to 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850 or 1900 consecutive nucleotides of a polynucleotide as claimed in claim 1.

4. The polynucleotide as claimed in claim 2 or 3, which is isolated from a bacterium of the genus *Streptomyces*.

5. The polynucleotide as claimed in 2, 3 or 4, which encodes a protein involved in the biosynthesis of a macrolide.

6. The polynucleotide as claimed in claim 2, 3, 4 or 5, which encodes a protein having an activity similar to the protein encoded by the polynucleotide with which it hybridizes or with which it exhibits the identity.

7. A polypeptide resulting from the expression of a polynucleotide as claimed in claim 1, 2, 3, 4, 5 or 6.

8. A polypeptide involved in spiramycin biosynthesis, wherein the sequence of said polypeptide is:

(a) one of the sequences SEQ ID No. 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 27, 29, 31, 32, 33, 35, 37, 38, 39, 41, 42, 44, 46, 48, 50, 51, 52, 54, 55, 56, 57, 58, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 108, 110, 112, 114, 116, 117, 119, 121, 142, 144, 146, 148 and 150,

(b) one of the sequences as defined in (a), except that throughout said sequence, one or more amino acids have been substituted, inserted or deleted without affecting the functional properties thereof,

(c) one of the sequences consisting of the variants of the sequences (a) and (b).

9. A polypeptide exhibiting at least 70%, 80%, 85%, 90%, 95% or 98% amino acid identity with a polypeptide comprising at least 10, 15, 20, 30 to 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620 or 640 consecutive amino acids of a polypeptide as claimed in claim 8.

10. The polypeptide as claimed in claim 9, which is isolated from a bacterium of the genus *Streptomyces*.

11. The polypeptide as claimed in claim 9 or 10, which encodes a protein involved in the biosynthesis of a macrolide.

12. The polypeptide as claimed in claim 9, 10 or 11, which has an activity similar to that of the polypeptide with which it shares the identity.

13. A recombinant DNA, which comprises at least one polynucleotide as claimed in one of claims 1, 2, 3, 4, 5 and 6.

14. The recombinant DNA as claimed in claim 13, wherein said recombinant DNA is included in a vector.

15. The recombinant DNA as claimed in claim 14, wherein said vector is chosen from bacteriophages, plasmids, phagemids, integrative vectors, fosmids, cosmids, shuttle vectors, BACs and PACs.

16. The recombinant DNA as claimed in claim 15, which is chosen from pOS49.1, pOS49.11, pOSC49.12, pOS49.14, pOS49.16, pOS49.28, pOS44.1, pOS44.2, pOS44.4, pSPM5, pSPM7, pOS49.67, pOS49.88, pOS49.106, pOS49.120, pOS49.107, pOS49.32, pOS49.43, pOS49.44, pOS49.50, pOS49.99, pSPM17, pSPM21, pSPM502, pSPM504, pSPM507, pSPM508, pSPM509, pSPM1, pBXL1111, pBXL1112, pBXL1113, pSPM520, pSPM521, pSPM522, pSPM523, pSPM524, pSPM525, pSPM527, pSPM528, pSPM34, pSPM35, pSPM36, pSPM37, pSPM38, pSPM39, pSPM40, pSPM41, pSPM42, pSPM43, pSPM44, pSPM45, pSPM47, pSPM48, pSPM50, pSPM51, pSPM52, pSPM53, pSPM55, pSPM56, pSPM58, pSPM72, pSPM73, pSPM515, pSPM519, pSPM74, pSPM75, pSPM79, pSPM83, pSPM107, pSPM543 and pSPM106.

17. An expression vector, which comprises at least one nucleic acid sequence encoding a polypeptide as claimed in claim 7, 8, 9, 10, 11 or 12.

18. An expression system comprising a suitable expression vector and a host cell which allows the expression of one or more polypeptides as claimed in claim 7, 8, 9, 10, 11 or 12.

19. The expression system as claimed in claim 18, which is chosen from prokaryotic expression systems and eukaryotic expression systems.

20. The expression system as claimed in claim 19, which is chosen from systems of expression in the bacterium *E. coli*, baculovirus expression systems which allow expression in insect cells, expression systems which allow expression in yeast cells and expression systems which allow expression in mammalian cells.

21. A host cell into which at least one polynucleotide and/or at least one recombinant DNA and/or at least one expression vector as claimed in one of claims 1, 2, 3, 4, 5, 6, 13, 14, 15, 16 and 17 has been introduced.

22. A method of producing a polypeptide as claimed in claim 7, 8, 9, 10, 11 or 12, wherein said method comprises the following steps:

a) inserting at least one nucleic acid encoding said polypeptide into a suitable vector;

b) culturing, in a suitable culture medium, a host cell transformed or transfected beforehand with the vector of step a);

c) recovering the conditioned culture medium or a cell extract;

d) separating and purifying said polypeptide from said culture medium or else from the cell extract obtained in step c);

e) where appropriate, characterizing the recombinant polypeptide produced.

23. A microorganism blocked in a step of the biosynthetic pathway for at least one macrolide.

24. The microorganism as claimed in claim 23, which is obtained by inactivating the function of at least one protein involved in the biosynthesis of this (these) macrolide(s) in a microorganism which produces this (these) macrolide(s).

25. A microorganism as claimed in 24, wherein the inactivation of this (these) protein(s) is carried out by mutagenesis in the gene(s) encoding said protein(s) or by expression of one or more antisense RNAs complementary to the messenger RNA(s) encoding said protein(s).

26. The microorganism as claimed in claim 25, wherein the inactivation of this (these) protein(s) is carried out by mutagenesis via irradiation, by action of a mutagenic chemical agent, by site-directed mutagenesis or by gene replacement.

27. The microorganism as claimed in claim 25 or 26, wherein the mutagenesis or mutageneses is or are carried out *in vitro* or *in situ*, by suppression, substitution,

deletion and/or addition of one or more bases in the gene(s) under consideration or by gene inactivation.

28. The microorganism as claimed in claim 23, 24, 25, 26 or 27, wherein said microorganism is a bacterium of the genus *Streptomyces.*

29. The microorganism as claimed in claim 23, 24, 25, 26, 27 or 28, wherein the macrolide is spiramycin.

30. The microorganism as claimed in claim 23, 24, 25, 26, 27, 28 or 29, wherein said microorganism is a strain of *S. ambofaciens*.

31. The microorganism as claimed in claim 23, 24, 25, 26, 27, 28, 29 or 30, wherein the mutagenesis is carried out in at least one gene comprising a sequence as claimed in one of claims 1, 2, 3, 4, 5 and 6.

32. The microorganism as claimed in claim 25, 26, 27, 28, 29, 30 or 31, wherein the mutagenesis is carried out in one or more genes comprising one of the sequences corresponding to one or more of the sequences SEQ ID No. 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 28, 30, 34, 36, 40, 43, 45, 47, 49, 53, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 107, 109, 111, 113, 115, 118, 120, 141, 143, 145, 147 and 149.

33. The microorganism as claimed in claim 25, 26, 27, 28, 29, 30, 31 or 32, wherein the mutagenesis consists of the gene inactivation of a gene comprising a sequence corresponding to the sequence SEQ ID No. 13.

34. A strain of *Streptomyces ambofaciens*, which is a strain chosen from:

- one of the strains deposited with the Collection Nationale de Cultures de Microorganismes (CNCM) [National Collection of Cultures and Microorganisms] on July 10, 2002, under the registration number I-2909, I-2911, I-2912, I-2913, I-2914, I-2915, I-2916 or I-2917
- the strain SPM510.

35. A method of preparing a macrolide biosynthesis intermediate, which comprises the following steps:

a) culturing, in a suitable culture medium, a microorganism as claimed in one of claims 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34,

b) recovering the conditioned culture medium or a cell extract,

c) separating and purifying said biosynthesis intermediate from said culture medium or else from the cell extract obtained in step b).

36. A method of preparing a molecule derived from a macrolide, wherein a biosynthesis intermediate is prepared according to the method of claim 35, and the intermediate thus produced is modified.

37. The method of preparation as claimed in claim 36, wherein said intermediate is modified chemically, biochemically, enzymatically and/or microbiologically.

38. The method of preparation as claimed in claim 36 or 37, wherein one or more genes encoding proteins capable of modifying the intermediate by using it as substrate is (are) introduced into said microorganism.

39. The method of preparation as claimed in claim 36, 37 or 38, wherein the macrolide is spiramycin.

40. The method of preparation as claimed in claim 36, 37, 38 or 39, wherein the microorganism used is a strain of *S. ambofaciens*.

41. A microorganism which produces spiramycin I but which does not produce spiramycin II and III.

42. The microorganism as claimed in claim 41, which comprises all of the genes required for the biosynthesis of spiramycin I, but wherein the gene comprising the sequence SEQ ID No. 13 or one of its variants, or one of the sequences derived therefrom due to the degeneracy of the genetic code, and encoding a polypeptide of

sequence SEQ ID No. 14 or one of its variants, is not expressed or has been made inactive.

43. The microorganism as claimed in claim 42, wherein said inactivation is carried out by mutagenesis in the gene encoding said protein or by the expression of an antisense RNA complementary to the messenger RNA encoding said protein.

44. The microorganism as claimed in claim 43, wherein said mutagenesis is carried out in the promoter of this gene, in the coding sequence or in a noncoding sequence so as to make the encoded protein inactive or to prevent its expression or its translation therefrom.

45. The microorganism as claimed in claim 43 or 44, wherein the mutagenesis is carried out by irradiation, by the action of a mutagenic chemical agent, by site-directed mutagenesis or by gene replacement.

46. The microorganism as claimed in claim 43, 44 or 45, wherein the mutagenesis is carried out *in vitro* or *in situ*, by suppression, substitution, deletion and/or addition of one or more bases in the gene under consideration or by gene inactivation.

47. The microorganism as claimed in claim 41 or 42, wherein said microorganism is obtained by expressing the genes of the biosynthetic pathway for spiramycin without these genes comprising the gene comprising the sequence corresponding to SEQ ID No. 13 or one of its variants, or one of the sequences derived therefrom due to the degeneracy of the genetic code, and encoding a polypeptide of sequence SEQ ID No. 14 or one of its variants.

48. The microorganism as claimed in claim 41, 42, 43, 44, 45, 46 or 47, wherein said microorganism is a bacterium of the genus *Streptomyces*.

49. The microorganism as claimed in claim 41, 42, 43, 44, 45, 46, 47 or 48, wherein said microorganism is obtained from a starting strain which produces spiramycins I, II and III.

50. The microorganism as claimed in claim 41, 42, 43, 44, 45, 46, 47, 48 or 49, which is obtained by mutagenesis in a gene comprising the sequence corresponding to SEQ ID No. 13 or one of its variants, or one of the sequences derived therefrom due to the degeneracy of the genetic code, and encoding a polypeptide of sequence SEQ ID No. 14 or one of its variants having the same function.

51. The microorganism as claimed in claim 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50, wherein said microorganism is obtained from a strain of *S. ambofaciens* which produces spiramycins I, II and III, in which gene inactivation of the gene comprising the sequences corresponding to SEQ ID No. 13 or one of the sequences derived therefrom due to the degeneracy of the genetic code is carried out.

52. A strain of *S. ambofaciens*, which is the strain deposited with the Collection Nationale de Cultures de Microorganismes (CNCM) on July 10, 2002, under the registration number I-2910.

53. A method of producing spiramycin I, which comprises the following steps:

(a) culturing, in a suitable culture medium, a microorganism as claimed in one of claims 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 and 52,

(b) recovering the conditioned culture medium or a cell extract,

(c) separating and purifying the spiramycin I from said culture medium or else from the cell extract obtained in step b).

54. The use of a polynucleotide as claimed in one of claims 1, 2, 3, 4, 5 and 6, for improving the macrolide production of a microorganism.

55. A macrolide-producing mutant microorganism which has a genetic modification in at least one gene comprising a sequence as claimed in one of claims 1, 2, 3, 4, 5 or 6 and/or which overexpresses at least one gene comprising a sequence as claimed in one of claims 1, 2, 3, 4, 5 and 6.

56. The mutant microorganism as claimed in claim 55, wherein the genetic modification consists of a suppression, a substitution, a deletion and/or an addition of

one or more bases in the gene(s) under consideration, with the aim of expressing one or more proteins having greater activity or of expressing a higher level of this (these) protein(s).

57. The mutant microorganism as claimed in claim 55, wherein the overexpression of the gene under consideration is obtained by increasing the copy number for this gene and/or introducing a promoter which is more active than the wild-type promoter.

58. The mutant microorganism as claimed in claim 55 or 57, wherein the overexpression of the gene under consideration is obtained by transforming a macrolide-producing microorganism with a recombinant DNA construct as claimed in claim 13, 14 or 17, allowing the overexpression of this gene.

59. The mutant microorganism as claimed in claim 55, 56, 57 or 58, wherein the genetic modification is carried out in one or more genes comprising one of the sequences corresponding to one or more of the sequences SEQ ID No. 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 28, 30, 34, 36, 40, 43, 45, 47, 49, 53, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 107, 109, 111, 113, 115, 118, 120, 141, 143, 145, 147 and 149, or one of its variants, or one of the sequences derived therefrom due to the degeneracy of the genetic code.

60. The mutant microorganism as claimed in claim 55, 56, 57, 58 or 59, wherein the microorganism overexpresses one or more genes comprising one of the sequences corresponding to one or more of the sequences SEQ ID No. 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 28, 30, 34, 36, 40, 43, 45, 47, 49, 53, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 107, 109, 111, 113, 115, 118 and 120, or one of its variants, or one of the sequences derived therefrom due to the degeneracy of the genetic code.

61. The mutant microorganism as claimed in claim 55, 56, 57, 58, 59 or 60, wherein said microorganism is a bacterium of the genus *Streptomyces*.

62. The mutant microorganism as claimed in claim 55, 56, 57, 58, 59, 60 or 61, wherein the macrolide is spiramycin.

63. The mutant microorganism as claimed in claim 55, 56, 57, 58, 59, 60, 61 or 62, wherein said microorganism is a strain of *S. ambofaciens*.

64. A method of producing macrolides, which comprises the following steps:

(a) culturing, in a suitable culture medium, a microorganism as claimed in one of claims 55, 56, 57, 58, 59, 60, 61, 62, 63 and 64,

(b) recovering the conditioned culture medium or a cell extract,

(c) separating and purifying said macrolide(s) produced from said culture medium or else from the cell extract obtained in step b).

65. The use of a sequence and/or of a recombinant DNA and/or of a vector as claimed in one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 17, for preparing hybrid antibiotics.

66. The use of at least one polynucleotide and/or at least one recombinant DNA and/or at least one expression vector and/or at least one polypeptide and/or at least one host cell as claimed in one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 21, for carrying out one or more bioconversions.

67. A polynucleotide which is a polynucleotide complementary to one of the polynucleotides as claimed in claim 1, 2, 3, 4, 5 or 6.

68. A microorganism producing at least one spiramycin, which overexpresses:

- a gene which can be obtained by polymerase chain reaction (PCR) using the following pair of sequence primers:

5' AAGCTTGTGTGCCCGGTGTACCTGGGGAGC 3' (SEQ ID No. 138) and
5' GGATCCCGCGACGGACACGACCGCCGCGCA 3' (SEQ ID No. 139)

and, as matrix, the cosmid pSPM36 or the total DNA of *Streptomyces ambofaciens*,

- or a gene derived therefrom due to the degeneracy of the genetic code.

69. The microorganism as claimed in claim 68 or 90, which is a bacterium of the genus *Streptomyces*.

70. The microorganism as claimed in claim 68, 69 or 90, which is a bacterium of the species *Streptomyces ambofaciens*.

71. The microorganism as claimed in claim 68, 69, 70 or 90, wherein the overexpression of said gene is obtained by transformation of said microorganism with an expression vector.

72. A strain of *Streptomyces ambofaciens*, which is the strain OSC2/pSPM75(1) or the strain OSC2/pSPM75(2) deposited with the Collection Nationale de Cultures de Microorganismes (CNCM) [National Collection of Cultures and Microorganisms] Pasteur Institute, 25, rue du Docteur Roux 75724 Paris Cedex 15, France, on October 6, 2003, under the registration number I-3101.

73. A recombinant DNA which comprises:

- a polynucleotide which can be obtained by polymerase chain reaction using the following pair of sequence primers:
5' AAGCTTGTGTGCCCGGTGTACCTGGGGAGC 3' (SEQ ID No. 138) and
5' GGATCCCGCGACGGACACGACCGCCGCGCA 3' (SEQ ID No. 139) and, as matrix, the cosmid pSPM36 or the total DNA of *Streptomyces ambofaciens*,

- or a fragment of at least 10, 12, 15, 18, 20 to 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1460, 1470, 1480, 1490 or 1500 consecutive nucleotides of this polynucleotide.

74. The recombinant DNA as claimed in claim 73 or 91, which is a vector.

75. The recombinant DNA as claimed in claim 73, 74 or 91, which is an expression vector.

76. A host cell into which at least one recombinant DNA as claimed in one of claims 73, 74, 75 and 91 has been introduced.

77. A method of producing a polypeptide, wherein said method comprises the following steps:

a) transforming a host cell with at least one expression vector as claimed in claim 75;

b) cultivating, in a suitable culture medium, said host cell;

c) recovering the conditioned culture medium or a cell extract;

d) separating and purifying said polypeptide from said culture medium or else from the cell extract obtained in step c);

e) where appropriate, characterizing the recombinant polypeptide produced.

78. The microorganism as claimed in claim 51, wherein the gene inactivation is carried out by in-phase deletion of the gene or of a part of the gene comprising the sequence corresponding to SEQ ID No. 13 or one of the sequences derived therefrom due to the degeneracy of the genetic code.

79. The microorganism as claimed in one of claims 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 or 78, which also overexpresses:

- a gene which can be obtained by polymerase chain reaction using the following pair of sequence primers:
5' AAGCTTGTGTGCCCGGTGTACCTGGGGAGC 3' (SEQ ID No. 138) and
5' GGATCCCGCGACGGACACGACCGCCGCGCA 3' (SEQ ID No. 139) and, as matrix, the cosmid pSPM36 or the total DNA of *Streptomyces ambofaciens*,
- or a gene derived therefrom due to the degeneracy of the genetic code.

80. An expression vector, wherein the polynucleotide of sequence SEQ ID No. 47, or a polynucleotide derived therefrom due to the degeneracy of the genetic code, is placed under the control of promoter which allows the expression of the protein encoded by said polynucleotide in *Streptomyces ambofaciens*.

81. The expression vector as claimed in claim 80, which is the plasmid pSPM524 or pSPM525.

82. A strain of *Streptomyces ambofaciens* transformed with a vector as claimed in claim 80 or 81.

83) The microorganism as claimed in one of claims 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 78, 79 and 92, which also overexpresses the gene having the coding sequence SEQ ID No. 47 or a coding sequence derived therefrom due to the degeneracy of the genetic code.

84. The microorganism as claimed in claim 83, which is the strain SPM502 pSPM525 deposited with the Collection Nationale de Cultures de Microorganismes (CNCM) Pasteur Institute, 25 rue du Docteur Roux 75724 Paris Cedex 15, France, on February 26, 2003, under the registration number I-2977.

85. A method of producing spiramycin(s), which comprises the following steps:

(a) culturing, in a suitable culture medium, a microorganism as claimed in one of claims 68, 69, 70, 71, 72, 78, 79, 82, 83, 84, 90 and 92,

(b) recovering the conditioned culture medium or a cell extract,

(c) separating and purifying the spiramycins from said culture medium or else from the cell extract obtained in step b).

86. A polypeptide, the sequence of which comprises the sequence SEQ ID No. 112 or the sequence SEQ ID No. 142.

87. A polypeptide, the sequence of which corresponds to the sequence translated from the coding sequence:

- of a gene which can be obtained by polymerase chain reaction (PCR) using the following pair of sequence primers:

5' AAGCTTGTGTGCCCGGTGTACCTGGGGAGC 3' (SEQ ID No. 138) and

5’ GGATCCCGCGACGGACACGACCGCCGCGCA 3’ (SEQ ID No. 139) and, as matrix, the cosmid pSPM36 or the total DNA of *Streptomyces ambofaciens*,

- or of a gene derived therefrom due to the degeneracy of the genetic code.

88. An expression vector which allows the expression of a polypeptide as claimed in claim 86, 87 or 93 in *Streptomyces ambofaciens*.

89. The expression vector as claimed in claim 88, which is the plasmid pSPM75.

90. The microorganism as claimed in claim 68, wherein the gene which can be obtained by polymerase chain amplification is the gene of coding sequence SEQ ID No. 141, or a gene derived therefrom due to the degeneracy of the genetic code.

91. The recombinant DNA as claimed in claim 73, wherein the polynucleotide which can be obtained by polymerase chain amplification is a polynucleotide of sequence SEQ ID No. 141.

92. The microorganism as claimed in claim 79, wherein the gene which can be obtained by polymerase chain amplification is the gene of coding sequence SEQ ID No. 141, or a gene derived therefrom due to the degeneracy of the genetic code.

93. A polypeptide, the sequence of which is SEQ ID No. 142.